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(u) Isolation and Growth of Wild-type and Mutant Magnetotactic Bacteria

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Three habitats local to Durham were selected for this work; Mill Pond, a freshwater eutrophic pond draining Oyster River into Great Bay estuary, the estuary, and a marine coastal saltmarsh in which we have found large populations of sulfide-type magnetic bacteria. Data being sought will enable the first systematic comparison among marine, estuarine and freshwater habitats of seasonal and vertical distribution of natural populations of magnetotactic bacteria. Enrichments and pure cultures of facultative and obligate iron-reducing bacteria from these diverse environments have been made and are still in progress. To date, new strains of iron reducing bacteria, some of which may be magnetogenic, have been isolated but these have not been characterized. We now have a small collection (seven) strains of iron reducers in axenic culture and are working to improve methods for routine isolation of the marine forms (efforts being complicated by the abundance of sulfate reducers

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FINAL REPORT: CONTRACT N00014-90-J-1053
R&T CODE 4412073---01

DATE: December 17, 1990

CONTRACT TITLE: ISOLATION AND GROWTH OF WILD-TYPE AND MUTANT MAGNETIC BACTERIA

PRINCIPAL INVESTIGATOR: Dr. Richard P. Blakemore
Department of Microbiology

CONTRACTOR University of New Hampshire
Durham, New Hampshire 03824

PROJECT PERIOD 10/01/1989 to 09/30/1990

PROJECT GOALS

To isolate new magnetogenic and other iron-reducing bacteria from freshwater, estuarine and marine habitats and to improve the growth of *Aquaspirillum magnetotacticum* on solid media under conditions appropriate for magnetite formation.

RESULTS

(Numbers within parentheses refer to references cited and produced under the contract)

A long-term study of natural populations of magnetotactic bacteria was initiated. These included populations of a multicellular prokaryote which, unlike all previously described magnetotactic bacteria, produces intracellular magnetic iron sulfides (1, 2) rather than magnetite. Three habitats local to Durham were selected for this work; Mill Pond, a freshwater eutrophic pond draining Oyster River into Great Bay estuary, the estuary, and a marine coastal saltmarsh in which we have found large populations of sulfide-type magnetic bacteria. The data, the first of their kind, will enable the first systematic comparison among marine, estuarine and freshwater habitats of seasonal and vertical distribution of natural populations of magnetotactic bacteria.

Enrichments and pure cultures of facultative and obligate iron-reducing bacteria from these diverse environments have been made and are still in progress. To date, new strains of iron reducing bacteria, some of which may be magnetogenic, have been isolated but these have not been characterized. We now have a small collection (seven) strains of iron reducers in axenic culture and are working to improve methods for routine isolation of the marine forms (efforts being complicated by the abundance of sulfate reducers which promoted chemical reduction of the iron used). It is very significant that only three organisms representing this relatively recently described physiological group of dissimilatory iron reducers (all freshwater isolates) have been intensively studied. Thus, these and other isolates will be highly useful

to substantially broaden our understanding of bacterial dissimilatory iron reduction in marine habitats.

Conjugal transfer of plasmid RP4 to members of the genus *Aquaspirillum* was demonstrated. The potential cloning vectors RP4, pUCD2 and pSa151 were stably electrotransformed (via electroporation) into cells of *Aquaspirillum serpens, A. dispar* and *A. itersonii.* The results revealed the need to first (conjugally) cycle the RP4 plasmid DNA in spirilla prior to electrotransformation. This work (3) provides a basis for developing methods of gene transfer in the genus *Aquaspirillum*, eventually to include magnetotactic strains. The reported results offer a potential method of overcoming possible restriction and/or modification of DNA among *Aquaspirillum* species and are therefore of potential value in development of a gene transfer system in *A. magnetotacticum.*

Plans to obtain and characterize additional mutants of *A. magnetotacticum* strains MS-1 and MS-2 were deferred in favor of initiating collaborative research to phylogenetically characterize other magnetogenic bacteria, including *Bilophococcus magnetotacticus*, a species described by others from type material and studied intensively despite the fact that it has not been axenically cultured, is in progress. Phylogenetic characterization of magnetogenic bacteria was carried out with investigators at Indiana University. This involved nucleotide sequencing of the 16s rRNA gene of *A. magnetotacticum* strains MS-1 and MS-2. Using universal primers of PCR and sequence analysis provided from Dr. Norman Pace's laboratory, the PCR amplified and cloned gene was completely sequenced (dideoxynucleotide chain termination method). Corroborative evidence of 16s rRNA gene amplification was obtained through reverse transcription methods. Sequence comparisons were made with those in the Woese/Pace database enabling strain MS-1 to be placed with the α group of the proteobacteria (4,5). (From partial sequence information, strain MS-2 appeared identical to strain MS-1).

PUBLICATIONS SUPPORTED

1. Rodgers, F. G., R.P.Blakemore, N.A. Blakemore, R. B. Frankel, D.A.Bazylinski, D. Maratea, and C. Rodgers (1990) Intercellular structure in a many-celled magnetotactic prokaryote. Arch. Microbiol. *154*:18-22.

2. Rodgers, F. G., R.P.Blakemore, N.A. Blakemore, R. B. Frankel, D.A.Bazylinski, D. Maratea, and C. Rodgers (1990) Intercellular junctions, motility and magnetosome structure in a many-celled magnetotactic prokaryote. *In* R. B. Frankel and R. P. Blakemore (Eds.) Iron Biominerals, Plenum Press, New York.

3. Eden, P.A., and R. P. Blakemore (Accepted) Electroporation and conjugal plasmid transfer to members of the genus *Aquaspirillum.* Arch. Microbiol

4. Eden, P. A., T. M. Schmidt, R. P. Blakemore and N. R. Pace. (in press) Phylogenetic analysis of *Aquaspirillum magnetotacticum* using PCR-amplified 16s ribosomal RNA-specific DNA. *In* R. B. Frankel and R. P. Blakemore (Eds.) Iron Biominerals, Plenum Press, New York.

5. Eden, P. A., T. M. Schmidt, R. P. Blakemore and N. R. Pace. (accepted) Phylogenetic analysis of *Aquaspirillum magnetotacticum* using PCR-amplified 16s ribosomal RNA-specific DNA. Int. J. Syst. Bacteriol.

AWARDS AND HONORS

(None)

PERSONNEL SUPPORTED

This contract contributed partial support for the training of two Ph.D. graduate students

Peter A. Eden - male, caucasian
Frank A. Caccavo - male, caucasian

Partial support for a (female, caucasian) laboratory technician was also provided by the contract

APPENDICES

1. Project supported papers